

Gross nitrogen transformations and N₂O emission sources in sandy loam and silt loam soils

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Abstract: The soil type is a key factor influencing N (nitrogen) cycling in soil; however, gross N transformations and N₂O emission sources are still poorly understood. In this study, a laboratory ¹⁵N tracing experiment was carried out at 60% WHC (water holding capacity) and 25°C to evaluate the gross N transformation rates and N₂O emission pathways in sandy loam and silt loam soils in a semi-arid region of Heilongjiang Province, China. The results showed that the gross rates of N mineralization, immobilization, and nitrification were 3.60, 1.90, and 5.63 mg N/(kg·d) in silt loam soil, respectively, which were 3.62, 4.26, and 3.13 times those in sandy loam soil, respectively. The ratios of the gross nitrification rate to the ammonium immobilization rate (n/i_a) in sandy loam soil and silt loam soil were all higher than 1.00, whereas the n/i_a in sandy loam soil (4.36) was significantly higher than that in silt loam soil (3.08). This result indicated that the ability of sandy loam soil to release and conserve the available N was relatively poor in comparison with silt loam soil, and the relatively strong nitrification rate compared to the immobilization rate may lead to N loss through NO₃⁻ leaching. Under aerobic conditions, both nitrification and denitrification made contributions to N₂O emissions. Nitrification was the dominant pathway leading to N₂O production in soils and was responsible for 82.0% of the total emitted N₂O in sandy loam soil, which was significantly higher than that in silt loam soil (71.7%). However, the average contribution of denitrification to total N₂O production in sandy loam soil was 17.9%, which was significantly lower than that in silt loam soil (28.3%). These results are valuable for developing reasonable fertilization management and proposing effective greenhouse gas mitigation strategies in different soil types in semiarid regions.

Keywords: gross N transformation rates; ¹⁵N tracing; N₂O emission sources; sandy loam; silt loam; semi-arid region

1 Introduction

Soil N transformations are microbially mediated processes and are affected by a range of factors, such as moisture content, temperature, cultivation, soil type, and land use type (Hoyle et al., 2006; Lan et al., 2013; Zhu et al., 2019). Among these factors, the soil type has been considered to be one of the most important regulating factors. There are many studies about the effect of soil type on N (nitrogen) transformations via the investigation of net rates (Grünzweig et al., 2003; Tripathi and Singh, 2009; Yang et al., 2010). However, net mineralization or nitrification rates are

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only valuable for indicating the N availability for plants and cannot provide detailed information on the production and consumption processes of soil inorganic N (Murphy et al., 2003). Alternatively, gross N transformation rates quantified by the ^{15}N isotope-labelling technique can estimate the gross rates of N mineralization, nitrification, and immobilization, respectively, which provide deeper and fundamental understandings of the N cycle inherent in the soil (Müller et al., 2007).

N_2O is an important greenhouse gas that is involved in global warming and the destruction of the stratospheric ozone layer (Byrnes, 1990). Agricultural soil is an important source of N_2O emissions, being responsible for 70% of anthropogenic emissions (Kroeze et al., 1999). N_2O is mainly produced from two biological processes in soil, namely, nitrification and denitrification (Stevens et al., 1997). These two processes are considered to occur simultaneously due to soil heterogeneity, which allows for aerobic and anaerobic zones to coexist in soil aggregates (Steven et al., 1997). However, the magnitude of N_2O emissions derived from nitrification and denitrification depends on many factors such as temperature, moisture content, fertilizer, and soil type (Dobbie and Smith, 2001; Khalil et al., 2004; Pihlatie et al., 2004; Lan et al., 2013; Zhang et al., 2019; Castellano-Hinojosa et al., 2020; Li et al., 2020), among which soil type is regarded as one of the most important controllers. Although some studies have estimated the specific processes leading to N_2O emissions from different soils, the results were varied. For example, most studies showed that N_2O was mainly released via nitrification at 30% to 60% water filled pore space (WFPS) in agricultural soils (Steven et al., 1997; Pihlatie et al., 2004; Bateman and Baggs, 2005; Well et al., 2008; Lan et al., 2014). However, Zhu et al. (2011) reported that denitrification was the dominant source of N_2O emissions from agricultural soils at 50% water holding capacity (WHC), and Li et al. (2016) indicated that the contribution of the denitrification process to N_2O emissions was larger than 50% at 40% to 60% WFPS in a sandy loam soil. The discrepancy of these results could result from the differences in soil texture, pH, and the availability of C and N. However, the underlying mechanisms and regulations are still not well understood.

The semi-arid region of Heilongjiang Province is an important agricultural area of Northeast China, sandy loam and silt loam soils are two typical agricultural soil types distributed in this region, where maize crops are grown annually. Large amounts of N fertilizer have been applied every year to increase the crop yield (Wang et al., 2013), and excessive N inputs are of environmental concern as they can cause water pollution and greenhouse gas emissions. It is especially crucial to better understand the N transformations under these two typical soil types to optimize fertilizer management and increase N use efficiency. However, there are few studies on the characteristics of gross N transformations in the agricultural soils of the semi-arid region. In addition, since soil N_2O emissions are mainly controlled by nitrification and denitrification, the contribution of these two processes to N_2O emissions in soils are need to be studied to well understand the N_2O emission sources for different soil textures of the semiarid region, to accurately estimate the N_2O inventories and plan mitigation strategies.

In this study, the aims were (1) to elucidate the gross N transformation rates in sandy loam and silt loam soils, and (2) to assess the relative importance of nitrification and denitrification on N_2O emissions under aerobic conditions. To meet these objectives, the ^{15}N tracing technique was used under laboratory incubation conditions to identify the N_2O emission sources in the two agricultural soils with substantially different soil properties, and the gross N transformations were calculated with the FLUAZ model.

2 Materials and methods

2.1 Study site and soil sampling

The study sites were located in Qiqihar City ($45^{\circ}53' - 48^{\circ}56'\text{N}$, $122^{\circ}24' - 126^{\circ}41'\text{E}$), in southwest Heilongjiang Province, China, which has a typical temperate continental semiarid monsoon climate. Precipitation is rare and strong winds occur frequently in spring. The annual mean

temperature is 2.3°C, and the mean annual precipitation is 454.0 mm, approximately 70% of which falls between June and September (Jiang et al., 2014). Two types of soil samples were collected from agricultural fields in this study and were classified as sandy loam and silt loam soil, according to the soil texture classification system issued by International Society of Soil Science. The conventional cropping practice of the study field is the continuous cultivation of corn from May to October every year, with a mean annual N fertilizer application of approximately 750 kg N/hm². The soil types used for this study represent typical cultivated soils, as sandy loam soil and silt loam soil cover approximately 6.68% and 5.29%, respectively, of the local agricultural land area (Du et al., 1994).

Soil sampling was conducted after the crop harvest in October, 2018. For each soil type, three sites that were 100 m×100 m in area were chosen randomly as space replications. At each site, three plots (4 m×4 m) were randomly established, and four soil cores (5 cm in diameter) were collected at a depth of 0–20 cm from the 4 sampling quadrates (2 m×2 m) of each plot. A total of 12 soil cores from one replicated site were pooled to form a composite sample. The roots and plant litter in the soil were picked out by hand, and then all of the soils were passed through a 2-mm sieve and split into two subsamples. One soil subsample was stored at 4°C before being used for incubation in one week and the other was air-dried to measure the basic physical and chemical properties.

2.2 ¹⁵N tracing experiment

¹⁵N tracing experiment was carried out using the method of Lang et al. (2010) with some modifications. Briefly, there were two ¹⁵N labeled treatments (each with three replicates) in this study, ¹⁵N labeled ammonium (¹⁵NH₄NO₃) and ¹⁵N labeled nitrate (NH₄¹⁵NO₃) with a 5-atom% of ¹⁵N isotopic excess. For each of the studied soils, 30 g (dry weight basis) of fresh soil was weighed into 30 (2 ¹⁵N labeled treatments×3 replicates×5 sampling times=30) Erlenmeyer flasks (250 mL). Each ¹⁵N labeled NH₄NO₃ solution was added to 15 flasks at a volume of 1 mL to reach a final applied N concentration of 40 μg NH₄⁺-N/g soil or 40 μg NO₃⁻-N/g soil, and then the soil was adjusted to a water content of 60% WHC. The flasks were sealed with silicone rubber stoppers and incubated for 7 d at 20°C under dark conditions. During the incubation period, the stopper was removed for 30 min every day to maintain the aerobic condition of the soil, and the water lost through the process of evaporation was compensated as needed by adding deionized water via a pipette.

At day 0, 1, 3, 5, and 7 after the addition of the NH₄NO₃ solution, gas samples inside the flasks were collected. For each sampling time, three replicate flasks of each ¹⁵N labeled treatment were randomly selected and flushed three times with room air using a multiport vacuum manifold (for 4 min each time), and then immediately sealed with silicone rubber stoppers (with a gas collection port) for 24 h. When sampling, the headspace gas inside the flask was sufficiently mixed through repeated extracting and injecting for 5 times with a gas-tight syringe. Subsequently, a 20 mL volume of the headspace gas was collected and injected into 12.5 mL pre-evacuated vials to determine the concentration and the ¹⁵N isotopic excess of N₂O-N using an Agilent 7890 gas chromatogram (Agilent Technologies, USA) and a Finnigan MAT 253 mass spectrometer (Thermo Electron Corporation, USA), respectively.

After gas sampling, soil samples were extracted with 2 mol/L KCl solution (at a soil to water ratio of 1.0:2.5) by shaking for 1 h at 250 r/min with a mechanical rotary shaker. The solution was then filtered into 100 mL plastic bottles through filter paper and stored at 4°C to determine the concentration and ¹⁵N isotopic excess of NH₄⁺ and NO₃⁻ within one week. To analyze the concentrations and ¹⁵N isotopic excess of NH₄⁺-N and NO₃⁻-N in the labeled soil, we used the steam distillation method to separate NH₄⁺ and NO₃⁻. In detail, a 10-mL volume of the KCl extracted solution was first steam distilled with magnesium oxide to separate NH₄⁺, and then distilled again with Devarda's alloy to separate NO₃⁻. During the distillation process, NH₄⁺ and NO₃⁻ were liberated in the form of NH₃ and were absorbed in 5 mL of a H₂SO₄ solution (0.010 mol/L). To prevent the cross contamination of the ¹⁵N isotopic excess between samples, we distilled 20 mL of ethyl alcohol after each sample to clean the distillation system. The

concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were determined by titrating the superfluous H_2SO_4 with 0.010 mol/L NaOH. Afterward, the titrated solution was acidified with 0.005 mol/L H_2SO_4 to reach a pH of 3 to avoid microbial contamination and dried at 65°C in an oven until reaching constant weight. The dried matter was packed into a tin capsule used for the analysis of ^{15}N isotopic excess with a stable isotope mass spectrometer (Finnigan MAT 253 mass spectrometer, Thermo Electron Corporation, USA). The extracted soil was washed three times with 150 mL of deionized water to remove the residual KCl, and then the soil was dried at 65°C and fine ground with a ball grinder. Then, a portion of the soil was packed into a tin capsule to measure the concentration and ^{15}N isotopic excess of organic N with a CN analyzer (NA Series 2, Capillary Electrophoresis Instruments, Italy) linked with the stable isotope ratio mass spectrometer.

2.3 Soil property analysis

The soil samples were stirred quickly with a glass bar for 1 min after adding deionized water (at a soil to water ratio of 1.0:2.5), and then left to stand for 30 min to measure the pH of the soil suspension with a DMP-2 mV/pH detector (Quark Ltd., China). The soil WHC was determined after water logging the air-dried soil for 2 h and natural draining for 12 h (Fierer and Schimel, 2002). The particle size distribution was measured using the hydrometer method after dispersion of the soil with pyrophosphate for 12 h. The sand, silt, and clay contents are expressed as a percentage by mass of the dispersed soil. The soil organic carbon (SOC) was analyzed by titration with FeSO_4 after digestion of the soil using $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$, and the total N (TN) was analyzed via a semi micro Kjeldahl digestion with a K_2SO_4 , CuSO_4 , and Se power mixture as the catalysts. We determined water soluble organic carbon (WSOC) and water soluble organic N (WSON) according to Burford and Bremner (1975). The inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) in the air-dried soil was extracted with 2 mol/L KCl (at a soil to water ratio of 1.0:2.5) on a mechanical shaker for 60 min and then filtered. The concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the filtrate were determined using a continuous-flow analyzer (Skalar Analytical Corporation, Netherlands).

2.4 Calculation and statistical analysis

The gross rates of N transformations were calculated by the FLUAZ model (Mary et al., 1998). The calculations of this model are based on the isotopic dilution and enrichment principles. It uses a numerical fourth order Runge-Kutta algorithm with a variable time-step to solve the differential system, and a non-linear fitting program (based on Marquardt's algorithm) to calculate the unknown N transformation rates between the $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and organic N pools. Minimizing the mean weighted error (MWE) is used as a criterion instead of the sum of squares when optimal fitting of the experimental data, which accounts for the variance of the measurements and normalizing of various variables (Mary et al., 1998). In the present study, five gross N transformation rates were estimated by the FLUAZ model, gross N mineralization rate (m , mg N/(kg·d)), gross nitrification rate (n , mg N/(kg·d)), gross N immobilization rate (i , mg N/(kg·d)), NH_4^+ immobilization rate (i_a , mg N/(kg·d)), and NO_3^- immobilization rate (i_n , mg N/(kg·d)) from the measurements of the amounts and ^{15}N isotopic excesses of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and organic N. The time-weighted average rates for the whole incubation period were calculated in addition to the individual time interval rates. The N_2O flux was calculated according to Lang et al. (2011). The N_2O emission fractions derived from nitrification and denitrification were calculated as described by Steven et al. (1997). Within the two emission pools in their model, the contribution of denitrification (d) and nitrification ($1-d$) to the N_2O emissions could be calculated from the $\text{NH}_4^{15}\text{NO}_3$ labeled treatment, and the equation is shown as follows:

$$d = \frac{a_m - a_n}{a_d - a_n}, \quad a_d \neq a_n, \quad (1)$$

where a_m is the ^{15}N isotopic excess of the N_2O mixture (atom%); a_n is the ^{15}N isotopic excess of NH_4^+ (atom%); and a_d is the ^{15}N isotopic excess of NO_3^- (atom%).

Differences in the measured soil properties and the time-weighted average gross N transformation rates between sandy loam and silt loam soils were assessed by paired sample

t-tests. All of the statistical analyses were carried out by SPSS 13.0 (IBM, USA).

3 Results

3.1 Soil properties

As shown in Table 1, there were significant differences in soil properties between the sandy loam and silt loam soils. The sandy loam soil had a sand content of 80.5%, which was significantly higher than that of silt loam soil (23.4%), while the silt and clay contents in sandy loam soil were 11.1% and 8.40%, respectively, which were significantly lower than those in silt loam soil. The WHC, SOC, TN, WSOC, WSON, NH₄⁺, and NO₃⁻ contents in sandy loam soil were all significantly lower compared to those in silt loam soil, whereas no significant difference in pH between these two soils was found.

Table 1 Basic physical and chemical properties of the tested soils

Property	Sandy loam soil	Silt loam soil
pH	6.3±0.5 ^a	5.7±0.4 ^a
WHC (%)	39.3±3.2 ^b	55.5±3.9 ^a
SOC (g/kg)	14.6±2.6 ^b	21.4±2.1 ^a
TN (g/kg)	1.1±0.1 ^b	2.1±0.2 ^a
C/N ratio	12.9±2.0 ^a	10.3±1.0 ^a
WSOC (mg C/kg)	154.1±13.0 ^b	257.2±11.3 ^a
WSON (mg N/kg)	16.4±2.1 ^b	28.9±2.3 ^a
Sand (>50 μm) (%)	80.5±4.1 ^a	23.4±1.9 ^b
Silt (2–50 μm) (%)	11.1±0.7 ^b	62.3±2.1 ^a
Clay (<2 μm) (%)	8.4±0.4 ^b	14.3±2.1 ^a
NH ₄ ⁺ -N (mg/kg)	1.8±0.1 ^b	5.1±0.3 ^a
NO ₃ ⁻ -N (mg/kg)	9.7±1.5 ^b	14.8±1.4 ^a

Note: WHC, water holding capacity; SOC, soil organic carbon; C, carbon; N, nitrogen; TN, total N; WSOC, water soluble organic C; WSON, water soluble organic N. Different lowercase letters within the same row indicate significant different at *P*<0.05 level. Mean±SD; *n*=3.

3.2 Concentrations and ¹⁵N isotopic excess of inorganic N

For the two studied soils, there was an obvious decrease in the NH₄⁺ concentration and increase in the NO₃⁻ concentration with time, indicating that net nitrification occurred (Fig. 1). The decrease in the NH₄⁺ concentration and increase in the NO₃⁻ concentration were larger in the silt loam soil than in the sandy loam soil. In the ¹⁵NH₄NO₃ treatments for both soils, the ¹⁵N isotopic excess of NH₄⁺ decreased over time (Fig. 2a and b), indicating that the natural abundance of NH₄⁺ resulting from the mineralization of native organic N was continuously input into the NH₄⁺ pool. In the same ¹⁵N labeled treatment, the ¹⁵N isotopic excess of NO₃⁻ increased gradually during the whole incubation period due to the input of nitrified NH₄⁺ labeled with ¹⁵N. In the NH₄¹⁵NO₃ labeled treatments, the ¹⁵N isotopic excess of NH₄⁺ was very low and no significant change was observed between each sampling time (Fig. 2c and d), suggesting that for the ¹⁵N labeled NO₃⁻, the remineralization of the immobilized NO₃⁻ and the reduction of NO₃⁻ to NH₄⁺ could be neglected. Conversely, in the ¹⁵N labeled NO₃⁻ treatment, the ¹⁵N isotopic excess in the NO₃⁻ pool decreased over time, which was caused by the nitrification of the naturally abundant ¹⁵N containing NH₄⁺ pool (Fig. 2b and d). The ¹⁵N isotopic excess of NO₃⁻ in silt loam soil increased much faster than that in sandy loam soil in the ¹⁵NH₄⁺ spiked treatment (Fig. 2a and b), which was consistent with the faster decrease of the ¹⁵N isotopic excess of NO₃⁻ in silt loam soil than in sandy loam soil in the ¹⁵NO₃⁻-spiked treatment (Fig. 2c and d), indicating that nitrification was stronger in the silt loam soil.

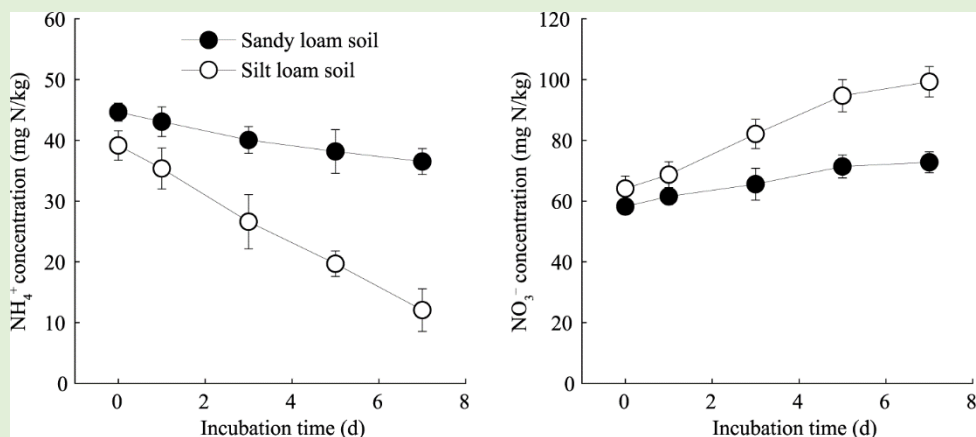


Fig. 1 Concentrations of NH_4^+ and NO_3^- in sandy loam and silt loam soils over the 7-d incubation time. Since the values of N (nitrogen) were equivalent for the ^{15}N labeled NH_4^+ and NO_3^- treatments, the data were pooled together. Vertical bars indicate standard deviations ($n=6$).

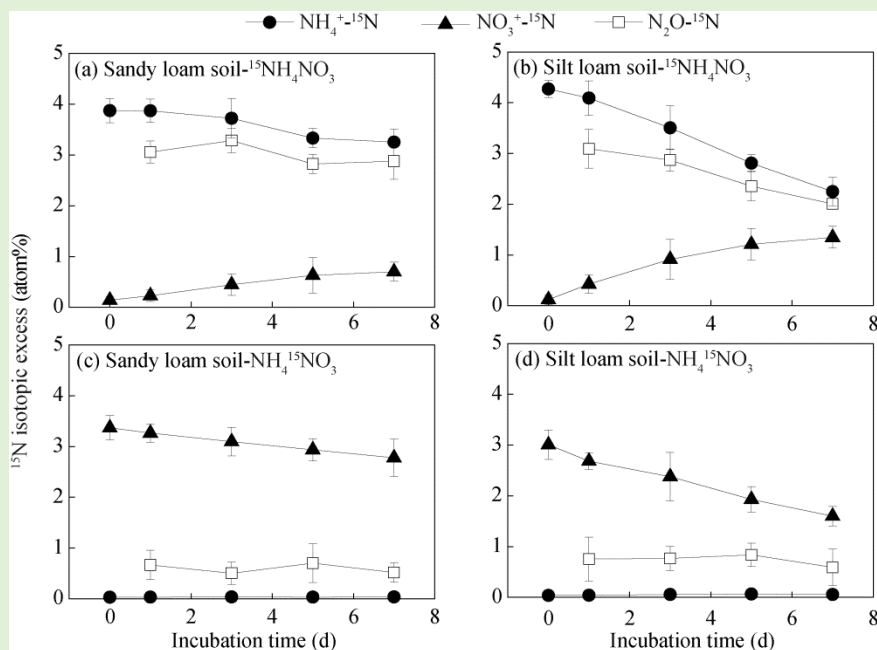


Fig. 2 ^{15}N isotopic excess of NH_4^+ , NO_3^- , and N_2O in the studied soils over the 7-d incubation time. (a), ^{15}N isotopic excess of sandy loam soil in the $^{15}\text{NH}_4\text{NO}_3$ treatment; (b), ^{15}N isotopic excess of silt loam soil in the $^{15}\text{NH}_4\text{NO}_3$ treatment; (c), ^{15}N isotopic excess of sandy loam soil in the $\text{NH}_4^{15}\text{NO}_3$ treatment; (d), ^{15}N isotopic excess of silt loam soil in the $\text{NH}_4^{15}\text{NO}_3$ treatment. Vertical bars indicate standard deviations ($n=3$).

3.3 Gross nitrogen transformations

For silt loam soil, the gross N mineralization rate reached its highest value during the first time interval and then decreased gradually until the end (Table 2). However, the gross N mineralization rate in sandy loam soil showed an initial increase until it peaked during the time interval of day 3 to 5 and declined thereafter. The average gross N mineralization rate in silt loam soil was 3.595 mg N/(kg·d), which was 3.62 times that in sandy loam soil (0.993 mg N/(kg·d)) (Fig. 3). Similar temporal patterns in the gross N immobilization rates were observed in sandy loam and silt loam soils, and were generally characterized by the highest rates during the first time interval and a subsequent decrease towards the end of the incubation period (Table 2). The average gross N immobilization rate in silt loam soil was 1.897 mg N/(kg·d), which was 4.26 times that in sandy loam soil (0.445 mg N/(kg·d)) (Fig. 3). The gross nitrification rate in sandy loam soil remained

nearly constant over the first 5 d, and then dropped sharply, whereas the gross nitrification rate in silt loam soil peaked during the time interval of day 1 to 3 and then decreased (Table 2). The average gross nitrification rate in silt loam soil was 5.632 mg N/(kg·d), which was 3.13 times that in sandy loam soil (1.796 mg N/(kg·d)) (Fig. 3).

From the results calculated by the FLUAZ model, we can see that both NH_4^+ and NO_3^- were immobilized in silt loam soil and sandy loam soil over the whole incubation period (Table 2). However, the immobilization rates of NH_4^+ were much higher than those of NO_3^- for each time interval in both soils (Table 2). The amount of NH_4^+ immobilized accounts for 70.2% to 98.0% of all immobilized N, demonstrating that microbes prefer to utilize NH_4^+ rather than NO_3^- .

Table 2 Gross N transformation rates in sandy loam and silt loam soils at different time intervals

Gross N transformation rate (mg N/(kg·d))	Soil type	Time interval			
		Day 0–1	Day 1–3	Day 3–5	Day 5–7
<i>m</i>	Sandy loam	0.133±0.071	0.289±0.051	2.831±0.427	0.289±0.061
	Silt loam	6.169±0.776	5.119±0.864	3.340±0.520	1.041±0.433
<i>i</i>	Sandy loam	0.980±0.104	0.377±0.107	0.280±0.051	0.412±0.024
	Silt loam	5.025±0.863	1.951±0.233	1.985±0.325	0.191±0.062
<i>i_a</i>	Sandy loam	0.917±0.090	0.350±0.124	0.258±0.063	0.377±0.068
	Silt loam	4.927±0.792	1.881±0.290	1.923±0.144	0.134±0.110
<i>i_n</i>	Sandy loam	0.064±0.014	0.027±0.004	0.023±0.014	0.035±0.024
	Silt loam	0.098±0.068	0.070±0.014	0.062±0.011	0.057±0.022
<i>n</i>	Sandy loam	2.198±0.331	2.089±0.411	2.273±0.313	0.826±0.091
	Silt loam	5.093±0.663	6.854±0.950	6.043±0.740	4.270±0.225

Note: *m*, gross N mineralization rate; *i*, gross N immobilization rate; *i_a*, NH_4^+ immobilization rate; *i_n*, NO_3^- immobilization rate; *n*, gross nitrification rate. Mean±SD; *n*=3.

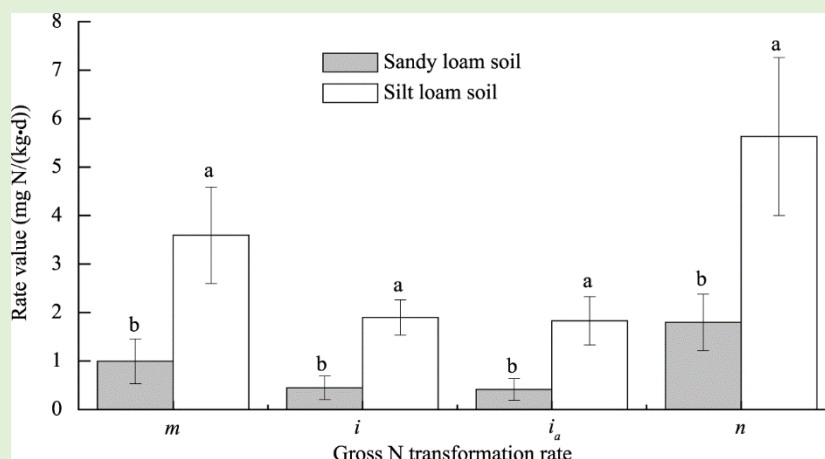


Fig. 3 Time-weighted average gross N transformation rates during day 0–7 in sandy loam and silt loam soils. *m*, gross N mineralization rate; *i*, gross N immobilization rate; *i_a*, NH_4^+ immobilization rate; *n*, gross nitrification rate. Vertical bars indicate standard deviations (*n*=3). Different lowercase letters on a pair of columns represent significant differences between the two soils at *P*<0.05 level.

The ratio of the gross nitrification rate to the NH_4^+ immobilization rate (*n*/*i_a*) could be used to indicate the dominant consumption pathway of NH_4^+ in the soil. For sandy loam soil, the ratio of *n*/*i_a* was 4.36, which was significantly higher than that of silt loam soil (3.08).

3.4 N₂O flux and sources

In silt loam soil, the N₂O flux increased sharply from day 1 to 3 and then declined quickly over the remaining incubation time. In sandy loam soil, the N₂O flux showed a slow increase from day 1 to 5, followed by a gradual decrease until the end of the incubation time (Fig. 4). For each sampling time, the N₂O flux in silt loam soil was significantly higher than that in sandy loam soil

($P < 0.01$). The average time-weighted N_2O flux over the incubation period in silt loam soil was $490 \text{ ng N}_2\text{O}/(\text{kg}\cdot\text{h})$, which was significantly higher than that in sandy loam soil ($174 \text{ ng N}_2\text{O}/(\text{kg}\cdot\text{h})$).

For the two studied soils, the ^{15}N isotopic excess of the N_2O pool was always between the ^{15}N isotopic excess of NH_4^+ and NO_3^- pool for both the $^{15}\text{NH}_4\text{NO}_3$ and $\text{NH}_4^{15}\text{NO}_3$ treatments (Fig. 2), and the ^{15}N isotopic excess of N_2O was closer to that of the NH_4^+ pool. This result demonstrated that N_2O was derived from both nitrification and denitrification processes, but nitrification led to more N_2O emissions than denitrification. The N_2O emission fraction derived from nitrification and denitrification varied with the sampling time (Fig. 5). The relative contribution of denitrification to N_2O emissions tended to first increase and then decrease with time in silt loam soil, whereas this value showed a pattern of decrease-increase-decrease in sandy loam soil. The opposite trend was observed for the relative contribution of nitrification to N_2O emissions in the two soils. For both soils, nitrification was the dominant source contributing to N_2O emissions, as the N_2O emission fraction derived from nitrification was significantly higher than that from denitrification over the whole incubation period (Fig. 5). In sandy loam soil, the average contribution of nitrification to the total N_2O production (82.1%) was significantly higher than that in silt loam soil (71.7%), and the average contribution of denitrification to total N_2O production was 17.9%, which was significantly lower than that in silt loam soil (28.3%).

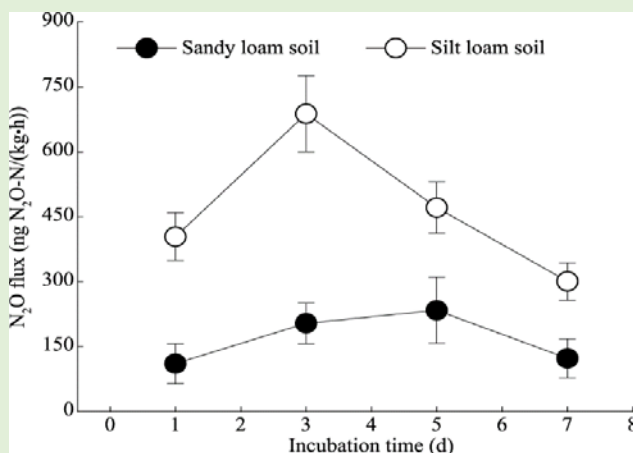


Fig. 4 N_2O flux from sandy loam and silt loam soils over the 7-d incubation time. Since the values of the N_2O flux were equivalent for the ^{15}N labeled NH_4^+ and NO_3^- treatments, the data were pooled together. Vertical bars indicate the standard deviations ($n=6$).

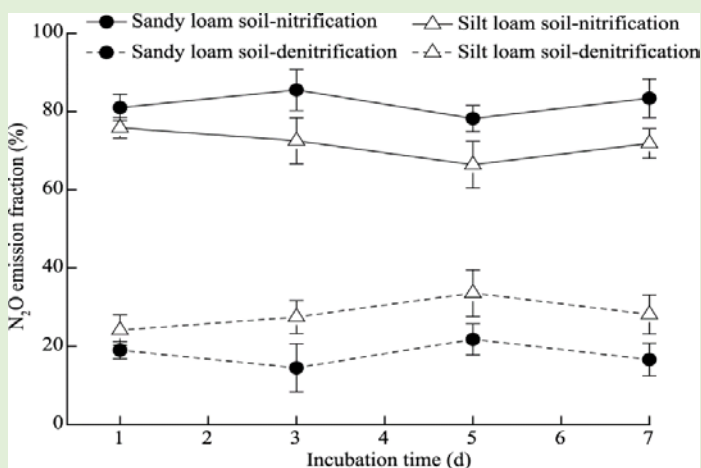


Fig. 5 N_2O emission fraction derived from the processes of nitrification and denitrification in sandy loam and silt loam soils over the 7-d incubation time

4 Discussion

4.1 Nitrogen transformation rates

Soil N transformations are microbially regulated processes that are influenced by a range of factors, among which soil type is regarded as one of the most important controllers (Cookson et al., 2007; Lang et al., 2010; Zhang et al., 2013; Zhu et al., 2019). Our research also supports this general assertion.

The present study showed that the gross N mineralization and immobilization rates were significantly lower in sandy loam than in silt loam soil. The reason for the lower rates of gross NH₄⁺ turnover may be due to the lower C (carbon) content in sandy loam soil (Table 1). It has been suggested that soil C could provide the substrate and energy for microbe growth in soil, as the positive correlations between SOC or WSOC and gross N turnover rates have been reported in many studies (Cookson et al., 2007; Lang et al., 2010, 2016; Zhang et al., 2013). Therefore, the higher contents of SOC and WSOC in silt loam compared to sandy loam soil may promote the growth and propagation of microorganisms and thus result in higher rates of N mineralization and immobilization. However, some studies showed that the gross N mineralization rate was more influenced by the SOC content in soil and was not affected by the quality of the substrate, while the immobilization process was mainly affected by the availability of soluble organic C (Hoyle et al., 2006), since the microbes participating in the process of mineralization could use a wide variety of complex organic substrates and microbes that dominate the immobilization process could only utilize simple organic carbohydrate (Gibbs and Barraclough, 1998). Thus, it is possible that the higher gross N mineralization and immobilization rates in silt loam compared with sandy loam soil could be attributed to the higher contents of SOC and WSOC, respectively, in silt loam soil.

In accordance with gross N mineralization and immobilization rates, gross nitrification rate in sandy loam soil was also lower than that in silt loam soil. In view of the similar pH of sandy loam and silt loam soils, some other factors such as NH₄⁺ availability and SOC may regulate the nitrification process in these two soils (Hart et al., 1994). Kuroiwa et al. (2011) reported strong correlations between the gross nitrification and mineralization rates and soil C concentrations, which was consistent with the results in this study, suggesting that the quantity of SOC controls the gross nitrification rate by regulating the gross mineralization rate, which engenders the available NH₄⁺ for the nitrifiers. The relatively lower SOC content and gross N mineralization rate in sandy loam compared to silt loam soil may therefore contribute to the lower gross nitrification rate in the former. In addition to SOC, soil texture may be another crucial parameter influencing gross nitrification (Pereira e Silva et al., 2012). Gross nitrification rates were found to correlate positively with the clay content and negatively with the sand content (Elrys et al., 2020), which is in agreement with our findings. The lower gross nitrification rate in sandy loam soil than in silt loam soil may be attribute to the following reasons. First, little available NH₄⁺ is produced via the exchange equilibrium in sandy soil (Aulakh et al., 1996). Second, the abundance and diversity of nitrifying bacteria is high in soil with larger clay content (Hosny, 1979; Elrys et al., 2020). Third, the cation exchange capacity of soil rich in clay is strong, thus the NH₄⁺ concentration in the soil solution can be reduced to levels that are nontoxic for microorganisms (Lam et al., 2018). Our results highlight that the soil texture and SOC content maybe the dominant factors controlling the gross nitrification rates in sandy loam and silt loam soils.

In soils under laboratory incubation conditions, nitrification and immobilization are the two main consumption fates for NH₄⁺ in soil. The ratio of the gross nitrification rate to the ammonium immobilization rate could reflect the dominant pathway of NH₄⁺ consumption and usually be used as an effective indicator for assessing NO₃⁻ leaching (Stockdale et al., 2002; Murphy et al., 2003). In this study, the n/i_a ratio of sandy loam soil (4.36) and silt loam soil (3.08) were both higher than 1.00, suggesting a very strong risk of NO₃⁻ leaching in these two soils. However, the significantly low rates of gross N mineralization and immobilization and high ratio of n/i_a in sandy loam soil indicated that sandy loam soil has relatively weak capacity for the production and conservation of available N and is more vulnerable for NO₃⁻ leaching in comparison with silt loam soil.

4.2 N₂O emission pathways

Our study showed that N₂O emissions from silt loam soil was significantly higher than that from sandy loam soil, which is in agreement with the previous findings of Pihlatie et al. (2004) and Syväsalö et al. (2004), who indicated that the N₂O in soils was larger in clay than in loamy sand soil. The high N₂O emission in silt loam soil could probably be attributed to the high contents of C and N, which could provide enough substrates for nitrification and denitrification. Furthermore, the significantly high clay content in silt loam soil may be favorable to build up anaerobic microsites, creating conditions conducive to denitrification processes and more N₂O emissions (Smith, 1980).

Under aerobic incubation conditions, nitrification was the dominant process in regulating the production of N₂O in soil; however, denitrification also plays an important role in N₂O formation. For example, Bateman and Baggs (2005) indicated that in agricultural soils with 60.0% and 50.0% WFPS, 18.5% and 23.9% of the N₂O was emitted from denitrification, respectively. Pihlatie et al. (2004) found that when the water content was adjusted to 60% WFPS in peat, clay, and loamy sand soils, the proportion of N₂O produced by denitrification reached a percentage of 22.0% to 33.0%. Lan et al. (2013) demonstrated that denitrification contributed to 16.3% and 23.9% of the total N₂O emission in two paddy soils when the water content was 65.0% WHC. In the present study, the average contribution of denitrification to the whole N₂O emission was 18.0% and 28.3% in sandy loam and silt loam soil, respectively, which was in accordance with these findings. The great contribution of denitrification to N₂O emissions suggests that denitrification occurred under aerobic conditions, even though the gross denitrification rates calculated by the FLUAZ model was close to zero in both soils (data not shown). The large amount of N₂O emission might be resulting from the formation of anaerobic microsites. Renault and Stengel (1994) pointed out that the fast growth of microbes consumed a large amount of oxygen in soil aggregates, leading to anoxic microenvironments. Additionally, anaerobic microsites may be formed in aerobic soils with high clay contents and high nitrification rates, since rapid nitrification would limit the supply of O₂ and a high clay content may prevent the diffusion of O₂ (Smith, 1980), resulting in anaerobic environments where denitrification can occur. Moreover, when the N solution was added into the soil via pipettes, the solution could not be distributed uniformly in the soil aggregates; thus, some aggregates may be saturated and conducive to denitrification. Garrido et al. (2002) also reported the occurrence of aerobic denitrification in soil, but the underlying mechanisms are still not well understood.

As discussed above, the low clay content and low rate of gross nitrification in sandy loam soil in comparison with silt loam soil (Table 1) may be the reason for the lower contribution of denitrification to N₂O emissions in sandy loam soil (17.9%) than in silt loam soil (28.3%). Our result is in agreement with Pihlatie et al. (2004), who found that the contribution of denitrification to N₂O emissions was lower in loamy sand (22.5%) than that in clay soil (33.5%) under aerobic conditions. Of the physical properties of mineral soils, the soil texture, macro- and micro-porosity, and soil aeration are important factors regulating the N₂O emission pathways (Simojoki and Jaakkola, 2000). Sandy loam soil would have larger macro-porosity, allowing for the transport and supply of oxygen, which would be unfavorable for the occurrence of denitrification in soil. Our results are supported by Pihlatie et al. (2004), who reported that nitrification was still the dominant process contributing to N₂O emission events at 100% WFPS for loamy sand soil that was visibly water logged. Castellano-Hinojosa et al. (2020) also showed that in a sandy loam soil incubated at 80% WFPS, N₂O was emitted mainly from nitrification rather than denitrification, indicating the good aerobic property of sandy loam soil, which is favorable for nitrification.

Our results are consistent with most published results, which show that nitrification was the main pathway to produce N₂O under aerobic conditions (Steven et al., 1997; Pihlatie et al., 2004; Well et al., 2008; Lan et al., 2014). In contrast, there are also some studies showed that denitrification was the dominant pathway of N₂O emission under aerobic conditions. For example, Zhang et al. (2011) indicated that denitrification accounted for 53.5% to 56.1% of the total N₂O production in four acidic forest soils incubated at aerobic conditions (40% to 52% WFPS). Li et al. (2016) reported that denitrification was estimated to contribute to more than 50% of N₂O emissions in loamy sand soil at 40% to 60% WFPS when adding crop residues. The possible reason behind the different result of Zhang et al. (2011) may be that fungi prevailed in acid forest soils, and the denitrification manipulated by fungi was an important N₂O emission source in

acidic forest soil due to the potential lack of the N₂O reductase in fungal biomass (Laughlin and Stevens, 2002). In the study of Li et al. (2016), the incorporation of crop residues resulted in the large consumption of oxygen, thus leading to the predominance of denitrification as a source of N₂O. As a result, the rates at which the two processes (nitrification and denitrification) dominate N₂O release under aerobic conditions are influenced by the soil type and substrate supply.

The results here indicated that the N₂O emission fraction from nitrification and denitrification fluctuated during the 7 d incubation time in sandy loam and silt loam soils, which is in agreement with Khalil et al. (2004), Li and Lang (2014) and Li et al. (2016), who also found the same phenomenon in unsaturated soils. However, Stevens et al. (1997) found that the contribution of denitrification to N₂O emissions decreased after two days of incubation, regardless of the water content in the soil. Additionally, in some cases, the N₂O emission fraction derived from denitrification increased with time under aerobic conditions (Wolf and Russow, 2000; Lan et al., 2013). The conflicting results between these studies may be related to the soil type, incubation time or availability of substrate, and the underlying mechanism needs further research.

4.3 Implications of the gross rates of N transformations and N₂O emission pathways

The sandy loam and silt loam soils selected for this study are typical for local agriculture in the western region of the Heilongjiang Province, China. The significant differences in these two soil properties lead to different rates of gross N transformations and N₂O emissions. Sandy loam soil had lower rates of gross N mineralization and immobilization than silt loam soil due to the relatively low C content, but the ratio of gross nitrification to ammonium immobilization rate was relatively high. This result suggests that mineral N produced from mineralization in sandy loam soil was low, therefore a large amount of N fertilizer is needed to meet the nutrient requirements of crop growth. However, it is better to apply the N fertilizer at low intensity and high frequency to avoid NO₃⁻ leaching, considering the low capacity of the soil to preserve the available N. In addition, this study showed that nitrification was the dominant process for emitting N₂O in both sandy loam soil and silt loam soil under aerobic conditions, and the contribution of nitrification to N₂O emissions in sandy loam soil was higher than in silt loam soil. These results provide an improved understanding to identify the relative importance between the two processes through which N₂O is produced in sandy loam and silt loam soils, which is valuable for improving the predictability of soil N₂O emissions, proposing N₂O mitigation strategies and establishing a calculation model to inventory greenhouse gases. However, the results were obtained under laboratory incubation conditions, which are different from the environmental variables found at a field site. Caution should be taken when extrapolating the results to field conditions.

5 Conclusions

Our study demonstrated that the soil type was an important factor influencing the gross N transformations and N₂O emissions from agricultural soil. Gross N mineralization, immobilization, and nitrification rates were significantly lower in sandy loam soil than in silt loam soil, but the ratio of gross nitrification rate to NH₄⁺ immobilization rate was much larger in sandy loam soil despite the ratio values were both higher than 1.00 in these two soils, suggesting that sandy loam soil had a relatively poor ability to supply and conserve the available N and was more vulnerable to NO₃⁻ leaching compared to silt loam soil. The processes of nitrification and denitrification took place simultaneously under 60% WHC for the two soils, nitrification was the dominant source of N₂O production and the contribution of nitrification to N₂O emissions was higher in sandy loam soil than in silt loam soil. These differences in gross N transformation rates imply that the soil type needs to be taken into consideration when manipulating fertilizer applications, and data on the pathway producing N₂O from different agricultural soils are valuable for proposing effective mitigation strategies and developing process-based models to reduce the uncertainties in estimating N₂O emissions. However, caution should be taken because this study was conducted under laboratory incubation conditions, and further research needs to be investigated at the field scale to take more factors into consideration.

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